Identifying the components in *Spl11*-mediated defense pathway and determining the relationship between *Spl11* and other defense signaling genes in rice

Gautam S. Shirsekar[1], Miguel E. Vega-Sanchez[1], Alicia Bordeos[2], Marietta Baraoidan[2], Hei Leung[2] and Guo-Liang Wang[1*] (*wang.620@osu.edu)

[1]Department of Plant Pathology, Plant Molecular Biology and Biotechnology Program, The Ohio State University, Columbus, OH 43210

[2]International Rice Research Institute (IRRI), DAPO box 7777, Metro Manila, Philippines

Abstract

Ubiquitination has recently been shown to be involved in programmed cell death (PCD) in plants. *Spl11* encodes an E3 ubiquitin ligase with U-Box and ARM repeat domains which negatively regulates PCD and disease resistance in rice. To identify new components in *Spl11*-mediated cell death pathway, a suppressor screen was performed using the *spl11* mutant GR5717 and EMS as a mutagen. The spontaneous lesion formation observed in GR5717 was found to be completely suppressed in one suppressor, whereas, two other suppressors showed partial suppression. Broad-spectrum resistance in GR5717 was found to be abolished in all the suppressors. To map the genes involved in this suppression phenomenon, three F2 mapping populations were generated using the suppressor lines and *spl11* mutant line TP309spl11. Two F2 populations show 3:1 ratio of segregation for suppression phenotype to lesion mimic phenotype whereas one shows 13:3 ratio, indicating either single-gene Mendelian inheritance or inheritance of one dominant and one recessive genes. Simultaneously F2 populations are also being generated to test for diallelism among different suppressors. Further, genetic analysis to study the relationship between *Spl11* and other defense signaling genes such as NPR1, SGT1, RAR1 and Rac1 has been undertaken. Crosses between *spl11* knockdown or *Spl11* overexpression lines and other defense mutants are being generated. Overall, ubiquitination mediated defense mechanisms will be elucidated through these studies in rice which is the world's most important food crop.

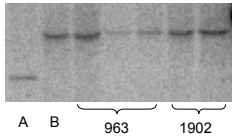
Introduction

Ubiquitination is emerging as an important cellular event which is involved in diverse biological processes in plants, such as development and responses to abiotic and biotic stresses (Vierstra, 2003; Zeng *et al.,* 2006). Identification of the *Spl11* gene's function in negative regulation of programmed cell death (PCD) in plants provided a direct link between PCD and /or disease resistance and ubiquitination. Cloning of *Spl11* by our lab has opened up the ubiquitination mediated PCD pathway for further in-depth analysis of genetic components involved in this pathway. Genetic suppressor screen approach has been used successfully by researchers to identify the interacting partners of a protein in question for their studies as well as dissecting a specific cellular pathway (Innes,1998; Kim and Delaney, 2002; Palma *et al.,* 2007 and Zhou *et al.,* 2008).In order to identify these genetic components of Spl11 regulated cell death we performed a suppressor screen using *spl11* mutant plants for further mutagenesis. Through such an effort we have identified three suppressor lines of lesion mimic phenotype which vary in their degree of suppression from full to partial.

Results

1 Identification of suppressor lines of spontaneous lesions caused by *spl11*

Suppressor screen was performed on GR5717 (containing *spl11* mutation) using EMS and one full suppressor line (963) and two partial suppressors (1902 and 6455) were generated which showed either no or highly reduced spontaneous lesion formation.

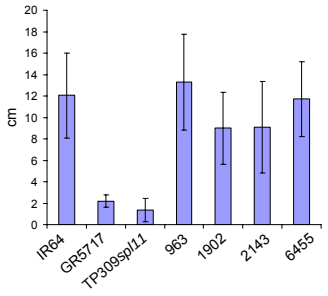


Further RFLP analysis followed by Southern blotting excluded the possiblity of *spl11* revertant mutations.

A. IR64 B. GR5717*spl11*

2 Response of suppressor lines to *Xanthomonas oryzae* pv. *oryzae* (Race 6)

A. 6455, B.2143, C.1902, D.963, E.IR64, F.GR5717



All the suppressor lines show loss of resistance to *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe grisea* (data not shown) which was typical to the lines carrying *spl11*mutation, suggesting probable role of those putative suppressor gene/s in cell death pathway related to pathogen resistance.

3 Genetic analysis of suppressor gene/s

In order to determine the number of genes involved in the phenomenon of suppression of spontaneous lesion formation and to initiate the mapping of the gene/s, we made crosses between TP309*spl11* and suppressor lines 963, 1902 and 6455. All the F_1 plants were suppressed suggesting dominant nature of the mutations. 3:1 (suppressed: non-suppressed lesion mimic) ratio was observed in F_2 population derived from crosses made between TP309*spl11* and 1902 and 6455, indicating inheritance of single dominant mutation in either of those suppressor lines. Ratio of 13:3 (suppressed :non-suppressed lesion mimic) was observed in F_2 progeny derived from cross between 963 and TP309*spl11*, suggesting inheritance of one dominant and one recessive mutation.

F_2 Population	Segregation ratio
TP309*spl11* X 1902	3:1 (p-value forX^2 =0.96)
TP309*spl11* X 6455	3:1 (p-value forX^2 =0.25)
963 X TP309*spl11*	13:3(p-value forX^2 =0.82)

Further these F_2 populations are being used for mapping the putative suppressor genes.

Future Directions

- The F_2 populations generated for the genetic analysis are currently being used for mapping the gene/s involved in the suppression phenomenon.
- We are currently testing F_2 populations generated from crosses among different suppressor lines to test for allelism between the gene/s involved in the suppression.
- In order to determine the role of *Spl11* in different defense related pathways in rice, we are currently creating double mutants by using *spl11* and mutant lines of various genes shown to be involved in defense pathways in rice such as *OsSGT1, OsRAR1, OsRac1, OsMapk5, OsBwmk1-1, OsEin2.* Also, we will be using lines over-expressing *OsNRR, OsNH1, OsRac1* to make crosses with *spl11* line. We have also generated a line over-expressing *Spl11* (TP309Ox*Spl11*) by *Agrobacterium tumefaciens* mediated transformation of rice. We will also be using this line to make the crosses with all the above mentioned lines for our studies.

Conclusions

- We identified three suppressor lines by performing suppressor screening on GR5717 (*spl11*) using Ethyl Methane Sulfonate as Mutagen
- All the suppressor lines show abolishment of resistance to *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe grisea*
- Suppressor line 1902 and 6455 show involvement of one dominant mutation in the suppression whereas line 963 shows involvement of one dominant and one recessive mutation manifesting in the suppression.

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